



#26 AF/1655

Patent  
Attorney's Docket No. 024705-083

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of: )  
Yoshihide HAYASHIZAKI ) Group Art Unit: 1655  
Application No.: 09/269,573 ) Examiner: B. J. Forman  
Filed: July 16, 1999 )  
For: METHODS FOR DETECTING )  
MUTATION IN BASE SEQUENCE )

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**BRIEF FOR APPELLANT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This appeal is from the decision of the Examiner dated October 2, 2001 (Paper No. 22), finally rejecting claims 1-25 and 27-33, which are reproduced as an Appendix to this brief. This brief has been revised for resubmission in response to the Notification of Non-Compliance with 37 C.F.R. § 1.192(c) mailed May 22, 2002 (paper no. 25).

No further fees are believed to be due by this paper. However, the Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.



Application No. 09/269,573  
Attorney's Docket No. 024705-083

**TABLE OF CONTENTS**

|                                                        | <b>PAGE</b> |
|--------------------------------------------------------|-------------|
| I. <u>Real Party in Interest</u> . . . . .             | 3           |
| II. <u>Related Appeals and Interferences</u> . . . . . | 3           |
| III. <u>Status of Claims</u> . . . . .                 | 3           |
| IV. <u>Status of Amendments</u> . . . . .              | 4           |
| V. <u>Summary of the Invention</u> . . . . .           | 4           |
| VI. <u>The Issues</u> . . . . .                        | 5           |
| VII. <u>Grouping of Claims</u> . . . . .               | 6           |
| VIII. <u>Argument</u> . . . . .                        | 7           |
| IX. <u>Conclusion</u> . . . . .                        | 13          |
| APPENDIX A . . . . .                                   | 14          |
| APPENDIX B . . . . .                                   | 20          |
| APPENDIX C . . . . .                                   | 21          |

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I. Real Party in Interest

The present application is assigned to The Institute of Physical and Chemical Research.

II. Related Appeals and Interferences

Neither the assignee nor their legal representative know of any other appeal or interferences which will affect or be directly affected by or have bearing on the Board's decision in the pending appeal.

III. Status of Claims

The Application was filed on March 30, 1999 with 30 claims, 4 of which were independent (claims 1, 9, 23 and 28). The filing date of July 16, 1999 was granted upon receipt of all requirements under 35 U.S.C. § 371. On October 10, 1999, an Official Action (Paper No. 7) was mailed rejecting claims 1-30.

On January 18, 2000, claim 26 was canceled and new claim 31 was added. Claims 1, 6, 8, 9, 13, 15, 16, 18, 21-25 and 27-30 were amended. On July 11, 2000 an Official Action (Paper No. 10) was mailed finally rejecting claims 1-25 and 27-31. On November 7, 2000, claims 1, 9 and 28 were amended. On November 27, 2000, amendments to claims 1, 9 and 28 were authorized by Dr. Malcolm McGowan via a telephonic interview with the Examiner. On January 7, 2001 an Advisory Action was mailed indicating that the amendments would be entered upon the payment of an extension of time fee and the filing of an appeal brief. The rejections of claims 1-25 and 27-31 over the cited art were maintained. A Notice of Appeal on behalf of all claims was filed on January 11, 2001.

On March 12, 2001 a Request for Continued Examination (RCE) was filed, with a Preliminary Amendment adding new independent claims 32 and 33. On March 23, 2001 an Official Action (Paper No. 19) was mailed rejecting claims 1-25 and 27-33. On June 25, 2001, a Reply and Amendment was filed. On October 2, 2001, an Official Action (Paper No. 22) was mailed finally rejecting claims 1-25 and 27-33.

On January 2, 2002 Appellant appealed the final rejection of claims 1-25 and 27-33.

The status of the claims as set out in Paper No. 22 was and is as follows:

allowed claims: none

claims objected to: none

claims rejected: 1-25 and 27-33 (Although the Office Action Summary and the Office Action on page 2 indicate that claims 1-25 and 27-30 are under prosecution, this is incorrect. The body of the Office Action rejects claims 1-25 and 27-33, all of which remain pending.)

IV. Status of Amendments

All amendments have been entered.

V. Summary of the Invention

Applicant's invention is directed toward methods for detecting mutations existing in nucleotide sequences by detecting mismatched base pairs. This invention is further directed to methods for detecting mutations existing in nucleotide sequences which can simultaneously detect expression levels of genes having the mutations. Specifically, this invention is directed to a method for detecting a fragment comprising fragments fixed on a substrate wherein the fragments have all of the sequence of a full-length gene (claims 1-22 and 32-33). The invention is further directed to a protein labeled with green fluorescence protein (GFP) (claims 23-27). The invention is further directed to an article comprising a substrate having a surface on which one or more fragments have all of the sequence of a full-length gene fixed in a hybridizable condition (claims 28-31).

Support for the invention can be found at least in the priority document JP 9-206602, filed on July 31, 1997. Support for the invention may be found in the specification on page 24, line 24 to page 6, line 13; page 9, lines 16 to 25; and page 4 line 22 to page 7, line 18 (the hybridization step of the present methods), page 7, line 19 to page 9, line 25 (the step of the present methods directed to the binding of a labeled protein), page 9, line 26 to page 11, line 33 (the step of the present methods directed to identifying a

fragment bound by the labeled protein), page 11, line 34 to page 12, line 32 (the step of the present methods directed to treating a mismatched base pair with a protein which recognizes and cleaves the mismatched base pair to cut the hybridized fragments at the mismatched base pair), page 12, line 33 to page 13, line 20 (the step of the present methods directed to labeling a fragment remaining on the substrate after the cleavage), and page 13, line 21 to page 15, line 17 (the step of the present methods directed to identifying the labeled fragment by detecting the label).

VI. The Issues

1. The Examiner has rejected claims 1-4, 19-21 and 28-32 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.*<sup>1</sup> This reference is appended hereto in Appendix B.
2. The Examiner has rejected claim 5 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Zoltukhin *et al.*<sup>2</sup> These are appended hereto in Appendix B.
3. The Examiner has rejected claims 6-8 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Gifford<sup>3</sup>. These are appended hereto in Appendix B.
4. The Examiner has rejected claims 9-18 and 33 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Chirikjian *et al.*<sup>4</sup> and Goldrick<sup>5</sup>. These

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<sup>1</sup>(WO 93/02216)

<sup>2</sup>(U.S. Patent No. 5,874,304)

<sup>3</sup>(U.S. Patent No. 5,750,335)

<sup>4</sup>(U.S. Patent No. 5,763,178)

<sup>5</sup>(U.S. Patent No. 5,891,629)

are appended hereto in Appendix B.

5. The Examiner has rejected claims 23-25 and 27 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Zoltukhin *et al.*<sup>6</sup> and Fleck *et al.*<sup>7</sup> These are appended hereto in Appendix B.

VII. Grouping of Claims

1. For the purposes of the rejection of claims 1-4, 19-21 and 28-32 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.*, it is the Applicant's intention that those claims stand or fall together.

2. Only claim 5 stands rejected under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Zoltukhin *et al.*

3. For the purposes of the rejection of claims 6-8 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Gifford, it is the Applicant's intention that those claims stand or fall together.

4. For the purposes of the rejection of claims 9-18 and 33 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Chirikjian *et al.* and Goldrick, it is the Applicant's intention that those claims stand or fall together.

5. For the purposes of the rejection of claims 23-25 and 27 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner *et al.* in view of Zoltukhin *et al.* and Fleck *et al.*, it is the Applicant's intention that those claims stand or fall together.

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<sup>6</sup>(U.S. Patent No. 5,750,335)

<sup>7</sup>(Nucleic Acid Research, 1994, 22:5289-5294)

VIII. Argument

1. *The Rejection of Claims 1-25 and 27-30 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner et al.*

The alleged teachings of Wagner *et al.* were set forth in the Official Action mailed October 2, 2001 (Paper No. 22). The Examiner asserts that Wagner *et al.* teach the use of a DNA with a hybridization partner "prepared using any know techniques and from any source, *e.g.*, naturally occurring DNA", and that it would have been obvious to the skilled artisan at the time the claimed invention was made to modify the DNA fixed onto the substrate of Wagner *et al.* by fixing a full-length gene sequence on the substrate for the benefit of detecting any and all mutations in a gene within a genomic sample (*See* Office Action, page 15). The Examiner further asserts that Wagner *et al.* disclose that there is no "upper limit on the size of the hybridization partner". Thus, the Examiner argues that it would be obvious to the skilled artisan at the time of the claimed invention to apply the unlimited length of the sequences of Wagner *et al.* to fix sequences having a full-length gene or cDNA for the benefit of detecting any and all mutations in a gene.

Applicant submits that this is not the case. For obviousness under §103, a reasonable expectation of success is required. In re Dow Chemical, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). One must inquire whether the prior art would have suggested to one of ordinary skill in the art that the claimed method should be carried out and would have a reasonable expectation of success, viewed in light of the prior art. Both the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure. Amgen Incorporated v. Chugai Pharmaceutical Company, Limited, 18 USPQ2d 1016, 1022 (Fed. Cir. 1991).

Applicant submits that the skilled artisan would not use the methods disclosed by Wagner *et al.* to prepare sequences having a full-length gene or cDNA because the skilled artisan could not have any expectation of success.

The method of Wagner *et al.* uses short oligonucleotides that are not full-length sequences for the detection of a mutation in a specific or known sequence. The flaws of this method include the fact that mutations or SNPs not included in the non-full-length

partner cannot be deleted. However, the presently claimed invention has unexpectedly shown that using a full-length hybridization partner will detect any mutation in any position.

Wagner *et al.* discloses the use of more nucleotide sequences as hybridization partners (see page 15, line 35) in the method that is commonly known as "tiling" (where more than one oligonucleotide sequence overlap, thus covering a longer sequence of the hybridization target than could be covered by a single fragment). In contrast, the method of the present invention uses full-length sequences, as opposed to the fragments of Wagner *et al.*. The present invention requires only one full-length sequence in order to be able to detect any mutation in the gene of interest. The method disclosed by Wagner *et al.* requires several oligonucleotides, and if the oligonucleotides do not cover the entire sequence of the gene, it is possible that mutations may be missed. Applicant again refers to the Declaration of Dr. Okazaki (originally filed with Applicant's response of June 25, 2001 and attached hereto as Appendix C) for further explanation of the difference between the method of claimed invention and that of Wagner *et al.*

The Examiner argues that Wagner *et al.* disclose that there is no upper limit on the size of the hybridization partner and thus, Wagner *et al.* inherently disclose a full-length fragment. However, Applicant submits that this is not the case. The skilled artisan would not think that the disclosure of Wagner *et al.* includes full-length sequences. Wagner *et al.* only disclose short partner sequences, and never disclose or suggest full-length sequences. Specifically, Wagner *et al.* disclose that the hybridization partner is about 20 to 100 nucleotides in length, and preferably, 20 to 40 nucleotides in length (p. 16, line 34 - p. 17, line 5). It would be illogical for the skilled artisan to extrapolate a full-length sequence for a hybridization partner, when the disclosure of Wagner *et al.* not only fails to disclose or suggest full-length sequences, but also teaches that short sequences of 20 to 40 nucleotides are preferable.

The Examiner notes that Wagner *et al.* teach the use of a DNA with a hybridization partner prepared using known techniques. However, the only "source" of known technique



that Wagner *et al.* cites by name is Sambrook *et al.*<sup>8</sup>. Specifically, in Example III, page 44, Wagner *et al.* state that a "cDNA corresponding to the p53 gene" is produced using the methods of Sambrook *et al.* Applicant notes that Sambrook *et al.* do not provide any protocol for or mention the preparation of full-length cDNAs as hybridization partners. Only a cDNA of the p53 gene has been prepared. Skill in the art cannot be used to supply missing knowledge of prior art to reach an obviousness judgement. The skill in the art does not act as a bridge over gaps in an obviousness case. See Al Site Corporation V. VSI International Inc., 174 F.3d 1308, 1318, 50 U.S.P.Q. 2d 1161, 1171 (CAFC 1999). There is no teaching or suggestion in Wagner *et al.* that a full-length cDNA has been prepared. Again, without a teaching of the use of full-length DNA, the skilled artisan would not be motivated to use a full-length DNA in the method of Wagner *et al.*

Moreover, Wagner *et al.* is non-enabling for the preparation of full-length sequences. A prior art reference must be enabling, thus placing the allegedly disclosed matter in the possession of the public. Akzo N.V. v. International Trade Commission, 1 USPQ 1241, 1245 (Fed. Cir. 1986). Because Wagner *et al.* specifically cite Sambrook *et al.* as the preferred protocol for preparing hybridization partners, and Sambrook *et al.* do not disclose or even suggest making a full-length sequence, the preparation of a full-length sequence is not enabled by Wagner *et al.*

The preparation of full-length cDNA requires particular skills and methods. At the time the invention was filed, and even today, it is considered difficult by those in the art to successfully obtain full-length cDNAs. Thus, without any clear motivation to prepare a full-length cDNA for hybridization, the skilled artisan, knowing the difficulty associated with preparing full-length cDNAs, would not attempt to prepare full-length cDNAs. Rather, they would prepare the small sequence fragments which Wagner *et al.* teach are preferable.

Wagner *et al.* disclose a hybridization method which requires the use of fragments. In fact, the skilled artisan would know that the method of Wagner *et al.* works more

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<sup>8</sup>Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, 2d Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989).

efficiently and accurately the more fragments are used. The presence of a full-length gene is inconsistent with the hybridization methodology of Wagner *et al.* Thus, the skilled artisan would not have any motivation nor any expectation of success, to modify the disclosure of Wagner *et al.* to arrive at the presently claimed invention. Accordingly, the presently claimed invention is not *prima facie* obvious over Wagner *et al.* The Applicant requests that this rejection be withdrawn.

2. *The rejection of claim 5 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner et al. in view of Zoltukhin et al.*

The requirements of a *prima facie* case of obviousness, and the deficiencies of Wagner *et al.*, are set forth in detail above. The Examiner cites Zoltukhin *et al.* for its teaching of GFP labeled proteins (Official Action at 7).

When all the prior art is considered together, a person having ordinary skill in the art must have a sufficient basis for the necessary predictability of success to sustain a rejection under 35 USC 103. *Ex parte Novitski*, 26 USPQ2d 1389, 1390 (BPAI 1993).

However, Zoltukhin *et al.* does not remedy the deficiencies of Wagner *et al.* and does not provide the expectation of success lacking in the primary reference. Specifically, Zoltukhin *et al.* neither discloses nor suggests the use of hybridization partners comprising the sequence of a full-length gene. Consequently, the presently claimed invention is not obvious over the combination of the cited references because these references neither disclose nor suggest all of the limitations of the presently claimed invention, as required by 35 USC §103. Withdrawal of this rejection is requested.

3. *The rejection of claims 6-8 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner et al. in view of Gifford.*

The requirements of a case of obviousness, and the deficiencies of Wagner *et al.*, are set forth in detail above. The Examiner cites Gifford for its teaching of a method

for detecting nucleic acid fragment having a mutation comprising:

hybridizing at least one fragment fixed on a substrate with at least on fragment of which mutation is to be assayed . . . and introducing a label into a fragment to be assayed to identify and quantify the fragment having a mismatch.

Official Action at 9. However, Gifford does not remedy the deficiencies of Wagner *et al.* Gifford neither discloses nor suggests the use of hybridization partners comprising the sequence of a full-length gene. Consequently, the presently claimed invention is not *prima facie* obvious over the combination of Wagner *et al.* and Gifford, because these publications neither disclose nor suggest all of the limitations of the presently claimed invention, as required by 35 USC §103. Withdrawal of this rejection is requested.

4. *The rejection of claims 9-18 and 33 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner et al. in view of Chirikjian et al. and Goldrick.*

The requirements of a *prima facie* case of obviousness, and the deficiencies of Wagner *et al.*, are set forth in detail above. The Examiner cites Chirikjian *et al.* for its teaching of a method

for detecting nucleic acid fragment having a mutation comprising:  
hybridizing nucleic acid fragments with nucleic acid fragments of which mutation is to be assayed treating a mismatched base pair occurring between the fragments with a substance specifically recognizing and cleaving the mismatched base pair labeling the cleaved fragments . . . and identifying the labeled fragment to thereby detect a nucleic acid having a mutation.

Official Action at 10. However, Chirikjian *et al.* do not remedy the deficiencies of Wagner *et al.* Specifically, Chirikjian *et al.* neither disclose nor suggest the use of hybridization partners comprising the sequence of a full-length gene.

The Examiner cites Goldrick for its teaching of a method

for detecting a mutation comprising: hybridizing a nucleic acid fragment with a fragment to be assayed; treating a mismatched base pair with a substance specifically recognizing and cleaving the mismatch base pair to cleave; and identifying the cleaved fragment to identify the mutated fragment wherein the cleaving substance is selected from S1 nuclease and Mung bean nuclease.

Official Action at 11-12. However, Goldrick does not remedy the deficiencies of Wagner *et al.* or Chirikjian *et al.* because Goldrick neither discloses nor suggests the use of hybridization partners comprising the sequence of a full-length gene.

Consequently, the presently claimed invention is not obvious over the combination of the cited references because these publications neither disclose nor suggest all of the limitations of the presently claimed invention, as required by 35 USC §103. Withdrawal of this rejection is requested.

5.     *The rejection of claims 23-25 and 27 under 35 U.S.C. § 103(a) as purportedly obvious over Wagner et al. in view of Zoltukhin et al. and Fleck et al.*

The requirements of a *prima facie* case of obviousness, and the deficiencies of Wagner *et al.*, are set forth in detail above. The Examiner cites Zoltukhin *et al.* for its teaching of GFP labeled proteins (Official Action at 14), and Fleck *et al.* for its teaching of "the MutS homologue of *Schizosaccharomyces pombe*, *swi4* which specifically binds to c°C mismatched base pairs" (Official Action at 14). However, neither Zoltukhin *et al.* nor Fleck *et al.* remedy the deficiencies of Wagner *et al.* Specifically, neither reference discloses nor suggests the use of hybridization partners comprising the sequence of a full-length gene. Thus, the presently claimed invention is not *prima facie* obvious over the combination of the cited references because these publications neither disclose nor suggest all of the limitations of the presently claimed invention, as required by 35 USC §103. Withdrawal of this rejection is requested.


IX. Conclusion

For the foregoing reasons, it is submitted that the Examiner's rejections of claims 1-25 and 27-30 were erroneous, and reversal of his decisions is respectfully requested.

Respectfully submitted,

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## APPENDIX A

### The Appealed Claims

1. (Three times Amended) A method for detecting nucleic acid fragment and/or PNA having a mutation, comprising the steps of:
  - (A) hybridizing at least one fragment among one or more fragments fixed on a substrate, which fragments are selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments and have all of a sequence of full-length gene, with at least one fragment of which mutation is to be assayed, wherein said fragment is selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments;
  - (B) binding a labeled protein, said protein specifically binding to a mismatched base pair occurring between the hybridized fragments having a mutation; and
  - (C) identifying a fragment bound by the labeled protein by detecting the label, thereby detecting a nucleic acid and/or PNA fragments having a mutation.
2. (Amended) The method of claim 1, wherein the protein specifically binding to a mismatched base pair is a mismatch binding protein.
3. The method of claim 2, wherein the mismatch binding protein is Mut S protein or analogue thereof, or a C<sup>o</sup>C mismatch binding protein.
4. (Twice Amended) The method of claim 1, wherein the protein specifically binding to a mismatched base pair is labeled with at least one kind of protein selected from the group consisting of luminescent proteins, phosphorescent proteins, fluorescent proteins, radioactive proteins, stable isotopes, antibodies, antigens, and enzymes.
5. (Twice Amended) The method of claim 1, wherein the protein specifically binding to a mismatched base pair is labeled with GFP (Green Fluorescence Protein).
6. (Twice Amended) The method of claim 1, wherein introducing a label into a

nucleic acid and/or PNA fragment to be assayed for mutations, and detecting the label of the nucleic acid and/or PNA fragment to be assayed for mutations, are carried out in order to identify and quantify the fragment having a mismatched base pair.

7. (Amended) The method of claim 6, wherein the label introduced into the nucleic acid and/or PNA fragment to be assayed for mutations produce a signal different from that produced by the label attached to the protein specifically binding to the mismatched base pair, and quantification and identification of the fragment having a mismatched base pair are simultaneously performed.

8. (Three times Amended) The method of claim 6, wherein the nucleic acid and/or PNA to be assayed for mutations is labeled with at least one kind of label selected from the group consisting of luminescent proteins, fluorescent proteins, phosphorescent proteins, stable isotopes, radioactive proteins, antibodies, antigens, and enzymes.

9. (Twice Amended) A method for detecting a nucleic acid fragment and/or PNA fragment having a mutation, comprising the steps of:

(A) hybridizing at least one fragment among one or more fragments fixed on a substrate, which fragments are selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments and have all of a sequence of full-length gene, with at least one fragment of which mutation is to be assayed, wherein said fragment is selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments;

(D) treating a mismatched base pair occurring between the hybridized fragments with a protein specifically recognizing and cleaving the mismatched base pair to cut the hybridized fragments at the mismatched base pair, or to remove at least a part of one strand of the fragments hybridized from the mismatched base pair;

(E) labeling a fragment remained on the substrate after the cleavage or removal; and

(F) identifying the labeled fragment by detecting the label, thereby detecting a nucleic acid and/or PNA fragment having a mutation.

10. (Amended) The method of claim 9, wherein said at least one fragment is fixed on the substrate at the 5' end and the 3' end of said fragment is blocked, and the labeling of the fragment in step (E) is performed by 3' end addition reaction.

11. (Twice Amended) The method of claim 9, wherein the protein specifically recognizing and cleaving the mismatched base pair is a nuclease.

12. The method of claim 11, wherein the nuclease is S1 nuclease, Mung bean nuclease or RNase H.

13. (Twice Amended) The method of claim 9, wherein the labeling of the fragment in the step (E) is performed by an enzyme reaction utilizing a label.

14. The method of claim 13, wherein the enzyme reaction is polymerase reaction, kination reaction, ligation reaction, or 3' end addition reaction.

15. (Three Times Amended) The method of claim 13, wherein the fragment is labeled with at least one kind of label selected from the group consisting of luminescent proteins, fluorescent proteins, phosphorescent proteins, stable isotopes, radioactive proteins, antibodies, antigens, and enzymes.

16. (Twice Amended) The method of claim 9, wherein introducing a label into a nucleic acid and/or PNA fragment to be assayed for mutations, and detecting the label of the nucleic acid and/or PNA fragment to be assayed for mutations, are carried out in order to detect and quantify the fragment having a mismatched base pair.

17. The method of claim 16, wherein the label introduced into the nucleic acid and/or PNA fragment to be assayed for mutations produce a signal different from that produced by the label attached to the fragment in the step (E), and quantification and identification of the fragment having a mismatched base pair are simultaneously performed.



18. (Three Times Amended) The method of claim 16, wherein the nucleic acid and/or PNA to be assayed for mutations is labeled with at least one kind of label selected from the group consisting of luminescent proteins, fluorescent proteins, phosphorescent proteins, stable isotopes, radioactive substances, antibodies, antigens, and enzymes.

19. (Amended) The method of claim 1, wherein the fragments of nucleic acid or PNA fixed on the substrate are bound to the substrate only at their 5' or 3' end.

20. (Amended) The method of claim 1, wherein the fragments of nucleic acid or PNA fixed on the substrate are fixed on the substrate by covalent bonds.

21. (Twice Amended) The method of claim 1, wherein said nucleic acid or PNA is cDNA.

22. (Twice Amended) The method of claim 9, wherein said nucleic acid or PNA is cDNA.

23. (Twice Amended) A protein specifically bindable to a mismatched base pair wherein said protein is labeled with GFP (Green Fluorescence protein).

24. (Twice Amended) The protein of claim 23, wherein the protein specifically bindable to the a mismatched base pair is a C°C mismatch binding protein.

25. (Twice Amended) A protein specifically bindable to a mismatched base pair, wherein said protein is a C°C mismatch binding protein.

27. (Three Times Amended) The protein of claim 25, wherein the label is at least one kind of label selected from the group consisting of luminescent proteins, phosphorescent proteins, fluorescent proteins, stable isotopes, radioactive proteins, antibodies, antigens, and enzymes.

28. (Twice Amended) An article comprising a substrate having a surface on which one or more kinds of nucleic acid or PNA fragments having all of the sequence of a full-length gene are fixed in a hybridizable condition.

29. (Amended) The article of claim 28, wherein said fragments fixed on the substrate are bound to the substrate only at their 5' or 3' ends.

30. (Twice Amended) The article of claim 28, wherein said fragments fixed on the substrate are bound to the substrate by covalent bonds.

31. The article of claim 28, wherein said nucleic acid or PNA is cDNA.

32. (Amended) A method for detecting nucleic acid and/or PNA having a mutation, comprising the steps of:

(A) providing

- at least one polynucleotide fixed on a substrate, wherein said polynucleotide has all of the sequence of full-length gene;
- a sample comprising at least one fragment of which mutation is to be assayed, wherein said fragment is selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments; and
- a labeled protein, wherein said protein specifically binds to a mismatched base pair resulting from hybridization between a polynucleotide and a fragment comprising a mutation;

(B) hybridizing said fragment to said polynucleotide;

(C) introducing said labeled protein under conditions that permit said protein to specifically bind to any mismatched base pairs that are present; and

(D) identifying a fragment bound by the labeled protein by detecting the label, thereby detecting a nucleic acid and/or PNA fragments having a mutation.

33. (Amended) A method for detecting a nucleic acid fragment and/or PNA fragment having a mutation, comprising the steps of:

(A) providing

- at least one polynucleotide fixed on a substrate, wherein said polynucleotide has all of the sequence of full-length gene; and
- a sample comprising at least one fragment of which mutation is to be assayed wherein said fragment is selected from the group consisting of one or more nucleic acid fragments and one or more PNA fragments;

(B) hybridizing said fragment to said polynucleotide;

(C) treating a mismatched base pair occurring between said hybridized fragment and said polynucleotide with a protein that specifically recognizes and cleaves a mismatched base pair to cut the hybridized nucleic acids at the mismatched base pair, or to remove at least a part of one strand of the nucleic acids hybridized from the mismatched base pair;

(D) labeling a polynucleotide remained on the substrate after the cleavage or removal; and

(F) identifying the labeled polynucleotide by detecting the label, thereby detecting a nucleic acid and/or PNA fragment having a mutation.

## APPENDIX B

### Cited Art

1. Chirikjian *et al.* (U.S. Patent No. 5,763,178)
2. Fleck (Nucleic Acid Research, 1994, 22:5289-5294)
3. Gifford (U.S. Patent No. 5,750,335)
4. Goldrick (U.S. Patent No. 5,891,629)
5. Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, 2d Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989).
6. Wagner *et al.* (WO 93/02216)
7. Zoltukhin *et al.* (U.S. Patent No. 5,750,335)
8. Zoltukhin *et al.* (U.S. Patent No. 5,874,304)

APPENDIX C

Declaration and Curriculum vitae of Okazaki Yasushi



29950014/1

Patent  
Attorney's Docket No. 024705-083

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of

HAYASHIZAKI

Application No.: 09/269,573

Filed: July 16, 1999

For: METHODS FOR DETECTING  
MUTATION IN BASE SEQUENCE

Group Art Unit: 1655

Examiner: B. Forman

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JUN 25 2002  
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**DECLARATION OF OKAZAKI YASUSHI**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, Okazaki Yasushi, hereby declare and state:

1. I am currently employed as Team Leader of the RIKEN Genome Science Laboratory, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa, Japan. My Curriculum Vitae is attached to this Declaration.
2. I have reviewed the above-cited patent application, and the PCT publication WO 03/02216 of Wagner et al. (WO 93/02216), and the U.S. Patent Examiner's statements regarding this publication in the Official Action mailed March 23, 2001, in connection with the above-cited application.
3. It is my understanding that the Examiner is arguing that the use of a full-length gene as a hybridization partner is implicit in the disclosure of the PCT publication. I gained this understanding in part from the following passage from page 4 of the Official Action:  

Wagner is silent with regard to the fragment having all of a full-length gene. However, the sequence of a full-length gene recited in Claim 1 is deemed to be inherent in the DNA hybridization partner having a mRNA target in Wagner et al. because DNA hybridization partners of mRNA inherently encompass a full-length gene and therefore the DNA hybridization partners of Wagner et al. encompass the sequence of a full-length gene.

(09/99)

4. I must disagree with this conclusion. The Wagner et al PCT publication states first, at page 6, lines 26-27, that the hybridization *partner* is cDNA or a synthetic oligonucleotide. Then, at page 6, lines 27-28, the Wagner et al. PCT publication states that the hybridization *target* is mRNA. It is clear to me from these passages that Wagner et al are explicitly distinguishing between hybridization "partner" and "target". I conclude from these passages that the hybridization partner in the method disclosed in the Wagner et al PCT publication is a cDNA or oligonucleotide fragment, and not a full-length gene.
5. From my knowledge of hybridization technology, and my review of the Wagner et al. PCT publication, I understand the reference to cDNA in Wagner et al. to refer to EST sequences or shotgun fragments, and not to full-length genes. In fact, cDNA generally used in the scientific community are fragments of cDNA since full-length cDNAs are difficult to prepare and require specific protocols.
6. I believe that this is confirmed in the Wagner et al PCT publication at Example III, page 44, and Example IV, page 46, where the preparation of the cDNA molecule used as hybridization partner (not target) are prepared by "standards methods" (Sambrook et al., 1989). I am familiar with standard methods, and with the Sambrook et al publication that discloses these methods. Such standard methods do not include the preparation of full-length cDNAs nor the use of full-length genes as hybridization partners.
7. It is also important to note that Wagner et al employ a "tiling" methodology in which several hybridization partner fragments overlapping with each other are fixed on a support in order to correspond (as a group) to the complete sequence of a full-length gene. In that method, the availability of short fragments as partners makes it possible to define the position of a mutated base, by observing which "tile" binds the mutated position. The "tiling" methodology therefore requires a high number of fragments (a high number of chips are needed in case of investigation of an entire genomic library) and a high number of mismatch-binding base proteins. In contrast, the method of the present invention allows the detection of a mismatched base in a target sample by using only one full-length DNA as a hybridization partner. This method, which is an "ON-OFF" method, can be used to

Immediately detect the presence or absence of a mutation and thereby allow diagnosis of a disease. In my opinion, the use of a full-length gene as hybridization partner is fundamentally incompatible with the tiling methodology carried out by Wagner. The presence of a full-length gene as hybridization partner is thus completely inconsistent with the use of fragments as hybridization partners. The "tiling methodology" and the "full-length" partner methodology are based on a different system, have different applications, and give different results.

8. I find no suggestion in the Wagner et al. PCT publication that would lead me to modify the method used in that publication by employing a full-length gene as a hybridization partner. Moreover, I know of no such suggestion outside of the disclosure of the above-cited application.

9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 23, 2001  
Date

  
Okazaki Yasushi, Ph.D.



## Curriculum vitae

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## Education (from Bachelor's Degree)

1980-1986 Medical Faculty of Okayama University, Medicine  
Awarded the degree of M.D.

1991-1995 Graduate School of Osaka University Medical School, Molecular Biology  
Awarded degree of Ph.D.

## Research Experience (list the most recent first)

08/99-Present: Senior Scientist  
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04/95-03/98 Research Scientist  
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10/92-03/95: Collaborator  
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## Clinical Experience

04/86-03/91: Cardiologist  
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## Publications

1. Kodama K., Okazaki Y., Nanto S., Mishima M., Hirayama A., Sato H., Kitakaze M., Hori M., Inoue M.: Possible Mechanism of the Beneficial Effects of Nitroglycerin in Patients with Effort Angina: Potential Roles of Collateral Circulation. in *Regulation of Coronary Blood Flow* M. Inoue, M. Hori, S. Imai, R.M. Berne (Eds.) (1991) Springer-Verlag 299-314
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#### Review

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